IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of: **Archer *et al.***) Examiner: **William O. Sandals**
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Application No. **09/446,681**)
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Filed: **14 March 2000**) Group Art Unit: **1636**
)
For: **Biosensor Materials and Methods**)

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DECLARATION OF JOHN ANTHONY CHARLES ARCHER

I, JOHN ANTHONY CHARLES ARCHER, hereby declare as follows.

1. I am presently employed as Senior Research Fellow at the University of Cambridge, Department of Genetics, Downing Site, Cambridge CB2 3EH.

2. My CV is appended to this declaration.

3. I am currently responsible for leading the work of two postgraduate students engaged in molecular genetic analysis of the Actinomycete *Rhodococcus.* This research applies molecular biology and functional genomics to investigate the genetic organisation and regulation of various genes involved in catabolism of nitro-aromatics and secondary metabolism.

4. I am a co-inventor of the subject matter covered in the above-referenced U.S. Patent Application Serial No. 09/446,681, entitled "Biosensor Materials and Methods" (referred to hereinafter as "the present application"), the pending claims of which are currently rejected in the U.S. Patent and Trademark Office.

5. I have read and am familiar with the Official Action dated 12 March 2002, in the present application. I understand the nature of the rejection made by the Examiner at point 11 of the Official Action concerning an alleged failure to meet the requirements of 35 USC 112 first paragraph. It is the Examiner's position that (absent evidence to the contrary) the specification does not provide teachings that the REG protein described in the application has regulatory activity towards a promoter of the *Rhodococcus ohp* operon. Further it is the examiner's position that the skilled artisan would have needed to have practiced considerable non-routine trial and error experimentation to practice the claimed invention. I disagree with these allegations for the reasons set forth in the following paragraphs.

6. Set out below are the results of experiments showing that the REG protein described in the present application switches on expression from a promoter of the ohp operon, located between the regulator and transport genes as described in the present application.

7. A standard expression assay was used to demonstrate function of OhpR/*Pohp/ohpO* regulation using a signal gene. In this technique, an easily assayed gene is placed under the transcriptional control of the test promoter/operator and its output measured in the presence or absence of the inducer compound in a genetic background that would not normally express the signal gene. The technique used was a derivative of a genetic test as taught by Miller (J. H. Miller, 1992, A short course in bacterial genetics : a laboratory manual and handbook for *Escherichia coli* and related bacteria, Cold Spring Harbor Laboratory Press). The technique places the signal gene (in our experiments, bacteral luciferase was used) under the transcriptional control of *Pohp/ohpO* region

and supplied *ohpR* in *cis* expressed from its own promoter; exogenous OHP (orthohydroxyphenylpropionic acid) was used as the inducer.

8. The *E.coli* vector pKB100, constructed in our laboratory, was used for these experiments. pKB100 is a pUC based cloning vector carrying the *Vibrio fischeri luxAB* genes (subcloned as a *BstYI* restriction fragment from pUC18X1f). The *luxAB* genes in pKB100 are oriented counter to the pUC *lacUV5* promoter and are under the transcriptional control of the *PohpR/ohpR/PohpABCD* regulator and promoter/operator region. The *PohpR/ohpR/PohpABCD* fragment used corresponds to nucleotides 1 to 1864 of the sequence shown in Figure 4 of the present application.

9. Functional testing of pKB100 luciferase expression was carried out in *E. coli* XL1 Blue. This host cannot utilise OHP as a carbon source, nor does the *E. coli* genome encode an OHP responsive regulatory protein. It is therefore a clean background to test *PohpRohpR/Pohp/ohpO* function. Similarly, the orientation of the *PohpRohpR/Pohp/ohpO-luxAB* gene cassette counter to the pUC *lacUV5* promoter means that expression of *ohpR* must be from its own promoter. Similarly, bacterial luciferase (*luxAB*) can only be expressed from the *Pohp*. pUC18X1f was used as a positive control, which uses the lacUV5 promoter to drive expression of the *luxAB* genes.

10. For each assay, a 10 ml LB culture supplemented with ampicillin (Amp) 100 micrograms per ml and or IPTG (pUC PlacUV5 inducer) or 2% glucose (pUC PlacUV5 repressor) or 5 mM OHP (Pohp inducer) was grown overnight at 37°C. 1 ml aliquots of each culture were placed in a cuvette and 5 microlitres of decanal (substrate for luxAB luciferase) added. Light output was measured in a Priors Scientific Instruments Mark1 photoluminometer. In a second experiment, to test the rapidity of OHP induction, OHP was added to uninduced 1 ml aliquots of pKB100 cultures (pKB100 cultured on LB + Amp) and incubated for 30 minutes at 37°C. Output from the photomultiplier tube was measured directly as current (mV) on addition of decanal. The results are shown below:

<u>Positive Control</u>		
pUC18X1f/LB Amp	-decanal = 0 mV	+ decanal = 230 mV
pUC18X1f/LB Amp + IPTG	-decanal = 0 mV	+ decanal = 255 mV
pUC18X1f/LB Amp + 2% glucose	-decanal = 0 mV	+ decanal = 0 mV
pUC18X1f/LB Amp + OHP	-decanal = 0 mV	+ decanal = 230 mV
<u>Test</u>		
pKB100/LB Amp + OHP	-decanal = 0 mV	+ decanal = 40 mV
pKB100/LB Amp + OHP + IPTG	-decanal = 0 mV	+ decanal = 40 mV
pKB100/LB Amp + OHP + 2% glucose	-decanal = 0 mV	+ decanal = 40 mV
<u>Rapid Induction Test</u>		
pKB100/LB Amp	-decanal = 0 mV	+ decanal = 17 mV
pKB100/LB Amp	-decanal = 0 mV	+ decanal = 17 mV +OHP 30 min= 35mV
pKB100/LB Amp	-decanal = 0 mV	+ decanal = 17 mV +2% glucose + OHP 30 min = 35 mV

11. These data show that pKB100 has a leaky expression of luciferase (17 mV) in the absence of OHP. This may be due to readthrough transcription from PohpR. However, clear evidence of induction is shown when pKB100 is cultured overnight in the presence of OHP (40 mV). This induction is independent of transcription from Plac as shown by the constant luciferase output in the presence of IPTG or glucose. To test the rapidity of OHP induction, OHP was added to pKB100 cultured only in the presence of Ampicillin or Ampicillin + glucose. Expression of

luciferase is enhanced approximately two fold by the addition of exogenous OHP, independent of IPTG and glucose. These data show that the region ohpR/Pohp encodes an OHP-sensitive regulatory element and promoter, and that expression from Pohp is driven by the OhpR (REG) protein.

12. Regarding the allegation that the demonstration that the OhpR (REG) protein interacts with the *ohp* operon promoter would require undue experimentation by one skilled in the art, I strongly disagree with this. Methods to determine whether or not a protein regulates expression from a particular promoter are routine and commonplace in the art. For one of ordinary skill in the art, no specific guidance over and above that given in the present application would be required to confirm the activity of the OhpR protein and *ohp* promoter.

13. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so make are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful statements may jeopardize the validity of the above-referenced application or any patent issued thereon.

John Anthony Charles Archer

DATE 9th Sept 2002 JOHN ANTHONY CHARLES ARCHER

CURRICULUM VITAE
JOHN A. C. ARCHER

I. Personal

Date of Birth	27th November 1959
Place of Birth	Bexleyheath, Kent, England
Citizenship	UK/US
Marital Status	Married
Home Address	36 West Field Little Abington Cambridge CB1 6BE UK

II. Current Position

Senior Research Fellow, Department of Genetics, Cambridge University, Cambridge CB2 3EH

III. Research Interests

Investigation and exploitation of *Rhodococcus* and *Nocardia* bacteria to develop molecule-specific sensors using genetically manipulated *Rhodococcus;* isolation, characterisation and metabolic engineering of novel catabolic enzymes and pathways.

IV. Commercial Experience

September 2000- present
Consultant Phico Therapeutics Ltd.

March 1999 - present
Co-founder and Director, Cambridge Microbial Technologies,

February 1997 - present
Co-founder and Technical Director, Pollution Technologies Ltd.

June 1989 - May 1991
Consultant Bio/Information Associates, Boston Massachusetts (Biodegradable Polymers).

V. Academic Experience

August 1991 - present
Senior Research Fellow, Department of Genetics, Cambridge University, Cambridge CB2 3EH

October 1985 - May 1991
Postdoctoral Fellow, Postdoctoral Associate, Department of Biology/BioProcess Engineering Center, The Massachusetts Institute of Technology, Cambridge, Massachusetts U.S.A.

October 1981 - October 1985
PhD. in Microbial Genetics, Department of Genetics, Church Street, Glasgow University. Supervisor Prof. David. J. Sherratt.

VI. Teaching and other Academic Experience

2001-present
Cambridge MIT Institute Fellow

1999 - 2000
Cambridge University, Senior Examiner, Medical and Veterinary Science Tripos IA (Medical Genetics) Cambridge University

1998 - 1999
Cambridge University, Examiner, Medical and Veterinary Science Tripos IA (Medical and Veterinary Genetics) Cambridge University

1995 - 1996
Cambridge University: Senior Examiner of Part II General Paper, Cambridge University
1994 - 1995
Cambridge University: Examiner of Part II General Paper, Cambridge University
1995 - present
Cambridge University: Supervision of PhD students (EC funded; BBSRC funded, NERC funded, SERC funded, Gates European Fellow Funding, MRC Bioinformatic Funding) Post-doctoral fellow (D. J. Fielding MRT funded) and numerous PartII Genetics Students.

1990 - 1991
Supervised Undergraduate Research Opportunities Student Senior (Senior Year) Department of Biology, Massachusetts Institute of Technology
1988 - 1990
Tutor, Massachusetts Institute of Technology

VII. Patent applications

6. US filing 9828660.2 Turk, J. N., Archer, J. A. C. 1998 "*Control of Gene Expression in Eukaryotes*"

5 US filing 9828661.2 Turk, J. N., Archer, J. A. C. "*Selectable Markers for Transformed Cells*"

4. UK filing Archer, J. A. C. 2000 "*Cell Encapsulation*"

3. US filing 09446,681 Archer, J. A. C., Powell, J. A. C., Jacquiau, H. R., Summers, D. K. "*Biosensor Materials and Methods*"

2. WO/9700517 Archer, J. A. C., Powell, J. A. C., Jacquiau, H. R., Summers, D. K. "*Biosensor Materials and Methods*"

1. US 788,114 Archer, J. A. C., Follettie, M. T., Sinskey, A. J. "*A deregulated homoserine dehydrogenase from Corynebacterium glutamicum for use in the production of L-threonine from bacteria.*"

VIII. Academic Publications

14. Fernandes, P. J. and Archer, J. A. C 2001 Construction of Rhodococcus random mutagenesis libraries using Tn5 transposition complexes *Microbiology*

13. Powell, J. A. C. and Archer, J. A. C. (1998). Molecular characterisation of a *Rhodococcus ohp* operon. Antonie Van Leeuwenhoek 74(1-3): 175-188.

12. Powell, J. A. C. and Archer, J. A. C. 1995 Genetic analysis of hydrocarbon degradation systems in *Rhodococcus corallina* V49. J. Cell.Biochem. 21:45.

11. Archer, J. A. C. and Griffiths, H. J.1995 Linear Plasmids in *Rhodococcus corallina* V49. J. Cell. Biochem. 21:51.

10. Archer, J. A. C. and Sinskey, A. J. 1993 The DNA sequence and minimal replicon of the *Corynebacterium glutamicum* plasmid pSR1: Evidence of a common ancestry with plasmids from *C. diphtheriae*. J. Gen. Microbiol. 139:1753-1759.

9. Archer, J. A. C., Sollow-Cordero, D. J. and Sinskey, A. J. 1992 A deletion in the carboxy terminus of *Corynebacterium glutamicum* homoserine dehydrogenase abolishes allosteric inhibition by L-threonine. Gene 107:53-59

8. Follettie, M.T., Peoples, O. P., Archer, J. A. C. and Sinskey, A. J. 1991. Metabolic Engineering of *Corynebacterium glutamicum*. pp 315-325 In: Proc. 6th Int. Symp. Genet. Industrial Microorganisms.

7. "Potential for Biotechnology in Bioremediation" 1991 , industrial consortium commissioned report Bio/Information Associates, Boston Massachusetts.

6. Marcel T., Archer, J. A.C., Mengin-Lecreulx, D. and Sinskey, A. J. 1990 Nucleotide sequence and organisation of the upstream region of the *Corynebacterium glutamicum lysA* gene. Mol. Microbiol. 4:1819-1829.

5. Han, K. S., Archer, J. A. C. and Sinskey, A. J. 1990 The molecular structure of the *Corynebacterium glutamicum* threonine synthase gene. Mol. Microbiol. 4: 1693-1706

4. Archer, J.A.C., M.T. Follettie, and A.J. Sinskey, Biology of *Corynebacterium glutamicum*: A molecular approach. Genetics and Molecular Biology of Industrial Microorganisms, 1989, Washington: American Society for Microbiology.

3. Boyd A. C, Archer, J. A. C, Sherratt, D. J. 1989 Characterisation of the ColE1 mobilisation region and its protein products. Mol. Gen. Genet. 217:488-498.

2. Peoples, O. P., Liebl, W., Bodis, M. Maeng, P. J., Follettie, M. T., Archer, J. A. C. and Sinskey, A. J. 1988 Nucleotide sequence and fine structural analysis of Corynebacterium glutamicum hom-thrB operon. Mol. Microbiol. 2:63-72.

1. Summers, D., Yaish, S., Archer, J. A. C., and Sherratt, D. J. 1985 Multimer resolution systems of ColE1 and ColK; localisation of the crossover site. Mol. Gen. Genet. 201:334-338.